3. Sekiguchi M, Hiroe M, Hiramitsu N. Natural history of acute viral or idiopathic myocarditis: a clinical and endomyocardial biopsy follow-up. In: Schultheiss HP, editor. New concepts of viral heart disease. Berlin: Springer; 1988. p. 33–50.
4. Bowles EN, Ni J, Kearney LD, Pauschinger M, Schultheiss HP, McCarthy R, et al. Detection of viruses in myocardial tissues by polymerase chain reaction: evidence of adenovirus as a common cause of myocarditis in children and adults. J Am Coll Cardiol. 2003;42:466–72.
5. Koide H, Kitaura Y, Deguchi H, Ukimura A, Kawamura K, Hirai K. Genomic detection of enteroviruses in the myocardium: studies on animal hearts with coxsackievirus B3 myocarditis and endomyocardial biopsies from patients with myocarditis and dilated cardiomyopathy. Jpn Circ J. 1992;56:1081–93.
6. Kotaka M, Kitaura Y, Deguchi H, Kawamura K. Experimental influenza A virus myocarditis in mice. Light and electron microscopic, virologic, and hemodynamic study. Am J Pathol. 1990;136:409–19.
7. Aoyama N, Izumi T, Hiramori K, Isobe M, Kawana M, Hiroe M, et al. National survey of fulminant myocarditis in Japan: therapeutic guidelines and long-term prognosis of using percutaneous cardiopulmonary support for fulminant myocarditis (special report from a scientific committee). Circ J. 2002;66:133–44.
8. Centers for Disease Control and Prevention (CDC). Patients hospitalized with 2009 pandemic influenza A (H1N1)—New York City, May 2009. MMWR Morb Mortal Wkly Rep 2010;58:1436–40.
9. Kerkhove MDV, Vandemaele KA, Shinde V, Jaramillo-Gutierrez G, Koukounari A, et al. on behalf of the WHO Working Group for Risk Factors for Severe H1N1pdm Infection. Risk factors for severe outcomes following 2009 influenza A (H1N1) infection: a global pooled analysis. PLoS Med 2011;7:1–12.
10. The Ministry of Health, Labor, Tokyo, Japan. A national survey of influenza patients in the last winter in Japan (in Japanese). http://www.mhlw.go.jp/bunya/kenkou/kekkaku-kansenshou04/pdf/110210-03.pdf. April 1, 2011.
11. Sugaya N. Widespread use of neuraminidase inhibitors in Japan. J Infect Chemother. 2011;17:595–601.
12. Ikematsu H, Kawai N, Kashiwagi S. In vitro neuraminidase inhibitory activities of four neuraminidase inhibitors against influenza viruses isolated in the 2010–2011 season in Japan. J Infect Chemother 2012 (published online Feb. 28).
13. Ukimura A, Izumi T, Matsumori A, and Clinical Research Committee on Myocarditis Associated with the 2009 Influenza A (H1N1) Pandemic in Japan organized by the Japanese Circulation Society. A national survey on myocarditis associated with the 2009 influenza A (H1N1) pandemic in Japan. Circ J 2010;74:2193–2199.
14. Arets HT. Myocarditis: the Dallas criteria. Hum Pathol. 1987;18:619–24.
15. Takeuchi I, Imaki R, Inomata T, Soma K, Izumi T. MRI is useful for diagnosis of H1N1 fulminant myocarditis. Circ J. 2010;74:2758–9.
16. Bratincsák A, El-Said HG, Bradley JS, Shayan K, Grossfeld PD, Cannavino CR. Fulminant myocarditis associated with pandemic H1N1 influenza A virus in children. J Am Coll Cardiol. 2010;55:928–9.
17. Weiss TW, Knut H, Stensaeth KH, Eritsland J. Myocarditis in a juvenile patient with influenza A virus infection. Eur Heart J. 2010;31:277.
18. Pan HY, Yamada H, Chida J, Wang S, Yano M, Yao M, et al. Up-regulation of ectopic trypsins in the myocardium by influenza A virus infection triggers acute myocarditis. Cardiovasc Res. 2011;89:595–603.
19. Wang S, Le TQ, Kurihara N, Chida J, Cisse Y, Yano M, Kido H. Influenza virus–cytokine-protease cycle in the pathogenesis of vascular hyperpermeability in severe influenza. J Infect Dis. 2010;202:991–1001.
20. Wang S, Le Quang T, Chida J, Cisse Y, Yano M, Kido H. Mechanisms of matrix metalloproteinase-9 upregulation and tissue destruction in various organs in influenza A virus infection. J Med Invest. 2010;57:26–34.
21. Teijaro JR, Walsh KB, Cahalan S, Fremgen DM, Roberts E, Scott F, et al. Endothelial cells are central orchestrators of cytokine amplification during influenza virus infection. Cell. 2011;146:980–91.

Hindawi Publishing Corporation
Influenza Research and Treatment
Volume 2012, Article ID 351979, 8 pages
doi:10.1155/2012/351979

Review Article

Myocarditis Associated with Influenza A H1N1pdm2009

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Received 14 June 2012; Revised 28 October 2012; Accepted 15 November 2012

Academic Editor: Daniel R. Perez

Acute myocarditis is a well-known complication of influenza infection. The frequency of myocardial involvement in influenza infection varies widely, with the clinical severity ranging from asymptomatic to fulminant varieties. The worst cases can result in death due to impaired cardiac function, although such fulminant myocarditis associated with influenza infection is rare, as shown by previous papers. Following the 2009 influenza pandemic, we reported on the clinical features of a cohort of 15 patients in Japan with H1N1pdm2009 myocarditis. In our subsequent survey of the literature for case reports or series of patients with myocarditis associated with H1N1pdm2009, we identified 58 detailed cases. We discuss here the high prevalence of fulminant myocarditis (36/58, 62%) among patients reported to have myocarditis associated with H1N1pdm2009. Mechanical circulatory support was required in 17 of the patients with fulminant myocarditis, 13 of whom recovered. We stress the need for increased awareness of influenza-associated myocarditis; such knowledge will facilitate earlier diagnosis and treatment of this fatal complication during future influenza pandemics.

1. Introduction

Acute myocarditis is a well-known complication of influenza infection. The clinical expression varies from asymptomatic to fulminant myocarditis, which can result in severe hemodynamic dysfunction, necessitating high-dose catecholamines and mechanical circulatory support [1–11]. Pathogens frequently associated with myocarditis include coxsackievirus and adenovirus; fulminant myocarditis resulting from influenza A viral infection is rare, as shown by previous literature [1–23]. Our interest in influenza-associated myocarditis follows from our experience with the influenza pandemic of 2009 [3, 24–30]. We surveyed the literature for case reports and series involving myocarditis associated with H1N1pdm2009, and identified 58 patients with such a diagnosis [3, 31–62]. In the present study, we review the clinical, laboratory, and pathologic characteristics of these 58 patients and theorize about the pathogenesis of influenza myocarditis [63–68].

2. Cardiac Involvement of Influenza Infection before the 2009 Pandemic

Myocarditis was a common and sometimes fatal complication of influenza infection in the pandemics of the previous century [1–7]. Small autopsy-based studies on fatal cases revealed a complication rate of focal to diffuse myocarditis of 39.4% during the 1957 Asian influenza pandemic and 48% during the Spanish influenza pandemic [4–6]. All of these fatal cases with myocarditis also had severe pneumonia and multiple organ involvement. Thus, myocarditis is likely to be a terminal complication of pandemic influenza infection.

On the other hand, while many people are affected by seasonal influenza every year, complications in nonrespiratory tissues (e.g., encephalopathy, myocarditis, and myopathy) occur only occasionally [1–7]. The frequency of myocardial involvement in influenza infection varies (0–10%) depending on the diagnostic criteria, and fulminant myocarditis associated with seasonal influenza infection is rare, as shown in previous papers [1–4, 9, 12, 13, 15–23]. Indeed, only two (2/505, 0.4%) myocarditis cases were reported in 505 children admitted with laboratory-confirmed influenza during the 2003/2004 season in Canada [16].

Only rarely are influenza viral antigens or genetic material detected in the myocardium. There has been only one case report in which seasonal influenza A RNA was detected in a myocardial biopsy [15]. Miura et al. detected viral antigen in the myocardium using immunohistochemical staining on an autopsied heart [18]. Bowles et al. screened

endomyocardial biopsy samples from 624 patients with clinically defined myocarditis using PCR for various viral genes. Among 239 samples that tested positive for viral genes, adenovirus was detected in 142 samples, enterovirus in 85 samples, and influenza A in only five samples (0.8%) [12]. Caforio et al. screened endomyocardial biopsy samples from 120 patients with histologically proven myocarditis using PCR to detect various viral genes. Among 31 samples that tested positive for viral genes, none contained influenza A or B virus (0%) [13]. Thus, the myocardial toxigenicity of the seasonal influenza virus seems to be rather weak.

3. Myocarditis Associated with Influenza H1N1pdm2009 in Japan

The Ministry of Health, Labor and Welfare (MHLW) of Japan confirmed only 198 deaths among about 20.61 million patients infected with influenza A H1N1pdm2009 in the pandemic season in Japan. They also confirmed that 15 of these deaths resulted from myocarditis associated with this pandemic strain [28, 29]. We previously reported 15 H1N1pdm2009 myocarditis patients and demonstrated their clinical features by conducting a cross-sectional national survey with assistance from all members of the Japanese Circulation Society (JCS) in the 2009/2010 influenza season [31]. Myocarditis was diagnosed using the Guidelines for Diagnosis and Treatment of Myocarditis (JCS 2009) [8]. Seven (47%) of the 15 myocarditis patients had no baseline disease. Myocarditis was proved by endomyocardial biopsy in six patients. Histological findings in these six patients included myocarditis with degenerated myocytes, infiltration of lymphocytes (ranging from mild to moderate, but not severe), and interstitial edema. We demonstrated a high prevalence of fulminant myocarditis with fatal arrhythmias and/or varying degrees of cardiogenic shock among the majority (10/15, 67%) of patients with myocarditis. Mechanical circulatory support with intra-aortic balloon pumping (IABP) and/or percutaneous cardiopulmonary support (PCPS) was emergently required in 10 patients. Eight of these 10 patients were successfully rescued with mechanical circulatory support, while the remaining two patients died. We demonstrated that, along with pneumonia and encephalopathy, myocarditis was an important cause of clinical deterioration in patients infected with the pandemic H1N1pdm2009 virus in Japan.

4. Myocarditis Associated with Influenza H1N1pdm2009 in the World

We reviewed the data of 58 patients (28 males and 30 females; mean age 32 years) with myocarditis associated with H1N1pdm2009 worldwide [3, 31–62] and identified a high prevalence of fulminant myocarditis (36/58, 62%) among them. The characteristics of these 58 myocarditis patients are summarized in Table 1. The mean age (32 years) of myocarditis patients associated with H1N1pdm2009 influenza was lower than the age of patients with seasonal influenza in the present study, indicating an age shift to a younger population in myocarditis patients during the pandemic [27–30, 32]. We speculate that the pathological mechanism of influenza myocarditis differs depending on the pathogen, and may depend on host immunity, as indicated by anti-H1N1pdm2009 titers.

Forty-two percent of these myocarditis patients had no baseline disease, and 23% had preexisting lung disease. The number of female patients was larger than the number of male patients, although general acute myocarditis is more common in males [69, 70]. Further, although pregnancy is reported to be a risk factor for deterioration of pandemic influenza infection, only one of the women in this paper was pregnant [38]. The mean interval from influenza onset to cardiac involvement was 5.4 days. Cardiac symptoms developed on the first to third day of sickness in 51% of myocarditis patients. Thirteen (24%) of the 58 cases were complicated by pneumonia. Most of these patients exhibited electrocardiogram (ECG) abnormalities, such as ST elevation (34%) and inverted T waves (24%). Fatal arrhythmias, such as ventricular fibrillation, ventricular tachycardia, and complete AV block, were recorded on the first day of hospitalization in 22% of the cases. Echocardiography revealed diffuse or focal left ventricular wall motion abnormalities in 90% of the patients. Mean ejection fraction was 25 ± 11%. Mortality rate was 24% (14 deaths/58 patients). Coronary studies were performed in 41% of these patients (64% of adult patients), all of which were normal with the exception of one case with a chronic total lesion. Myocarditis was proved by endomyocardial biopsy and/or autopsy in 14 patients. Myocardial biopsy did not contribute to the diagnosis of myocarditis in several cases. In the six patients in whom endomyocardial biopsy was performed, the pathological findings were mild even in clinically defined fulminant myocarditis patients, compared with general myocarditis patients reported in previous papers [71, 72]. Although immunohistology has been acknowledged to have a substantially higher sensitivity, we did not have detailed information on the immunohistological analysis of biopsies [73, 74].

Cardiovascular magnetic resonance imaging (CMR) was used as the diagnostic tool in several cases with pericardial/myocardial involvement during H1N1pdm2009 infection [47–50, 55, 58, 73–75]. A neuraminidase inhibitor (either oseltamivir, zanamivir, or peramivir) was used in 85% of the cases. A left ventricular assist device (LVAD) or PCPS was used in 10 cases, and IABP was used in 11 cases. Extracorporeal lung assist with extracorporeal membrane oxygenation (ECMO) was used in 12 cases. Mechanical circulatory support (PCPS or LVAD and/or IABP) was used in 17 of the patients with fulminant myocarditis, 13 of whom were rescued. Patchy hemorrhage was demonstrated in three autopsy cases. Reverse transcriptase polymerase chain reaction (RT-PCR) for H1N1pdm2009 from heart specimens tested positive in four cases [44, 51, 53, 58].

In the 2009 pandemic, the rate of cardiac complications seemed to be higher than that reported for seasonal influenza A virus infection. Randolph et al. reported that acute myocarditis associated with H1N1pdm2009 (1.4% of 838 cases) was an independent risk factor for death in children (<21 years old) admitted to a PICU in the USA [61].

Table 1: Detailed characteristics of 58 patients with myocarditis associated with H1N1pdm2009 influenza.

Characteristics of 58 patients with H1N1pdm2009 influenza reported in detail	Result (%)
Age (mean, years) (range)	32 (3–72)
Less than 17 years (%)	14 cases (24%)
Sex (% female)	30 cases (52%)
Death (%)	14 cases (24%)
Interval between influenza onset and cardiac symptoms (mean, days) (range)	5.4 (1–21)
1st day to the 3rd day (%)	51%
Cardiac symptoms	
Dyspnea (%)	54%
Chest pain (%)	30%
Fulminant myocarditis (%)	36 cases (62%)
Mortality rate of patients with fulminant myocarditis	39% (14/36)
Pneumonia as a complication (%)	13 cases (22%)
ECG findings on the first day of hospitalization	
ST elevation (%)	34%
T inversion (%)	24%
Fatal arrhythmias (VF, VT, complete AV block) (%)	22%
Echocardiogram	
Diffuse or focal left ventricular wall motion abnormalities	90%
Ejection Fraction (mean ± SD)	25 ± 11%
Percentage of patients in whom CAD was ruled out by CAG	41%
Percentage of adult patients in whom CAD was ruled out by CAG	64%
Treatment	
Neuraminidase inhibitors	85%
PCPS	10 cases (17%)
LVAD	1 case (1.7%)
IABP	11 cases (19%)
PCPS or LVAD and/or PCPS	17 cases (29%)
Mortality of patients treated with mechanical support	23% (4/17)
ECMO	12 cases (21%)
Biopsy	10 cases (17%)
Myocarditis with lymphocyte infiltration (mild~moderate)	6 cases
No myocarditis (according to the Dallas criteria)	4 cases
Autopsy	8 cases (14%)
Pachy hemorrhage in the autopsied heart	3/8 cases (38%)
RT-PCR positivity rate for H1N1pdm2009 virus from heart specimens	4 cases

ECG: electrocardiogram; VF: ventricular fibrillation; VT: ventricular tachycardia; AV block: atrioventricular block; CAD: coronary artery disease; CAG: coronary angiography; PCPS: percutaneous cardiopulmonary support; LVAD: left ventricular assist device; IABP: intra-aortic balloon pumping; ECMO: extracorporeal membrane oxygenation; RT-PCR: reverse transcription polymerase chain reaction.

Bratincsák et al. reported four patients with myocarditis associated with H1N1pdm2009 within a 30-day period in 2010 and suggested that H1N1pdm2009 virus might be more commonly associated with myocarditis than seasonal influenza virus [58]. Zheng et al. reported finding seven children (5%) with complicated myocarditis among 148 children hospitalized with influenza H1N1pdm2009 infection in China [62]. Shin et al. analyzed a group of 30 critically ill pediatric patients in Korea and reported that the most common causes of death were encephalopathy (four children) and myocarditis (four children) [63]. Martin et al. examined a cohort of 123 hospitalized patients infected with H1N1pdm2009 and reported that six patients (4.9%) had either new or worsened left ventricular dysfunction. They concluded that reversible cardiac dysfunction is a relatively common complication associated with H1N1pdm2009 [60]. Thus, the frequency of cardiac involvement in influenza virus infection is likely elevated with influenza H1N1pdm2009 compared to seasonal influenza.

5. Theories of Pathogenesis of Influenza Myocarditis

It is well known that coxsackieviruses present a high affinity for cardiac myocytes [9, 12–14]. There is a distinct difference in the pathological findings between myocarditis associated

with influenza A virus and myocarditis associated with coxsackieviruses [1–14]. The pathological effects of influenza viral myocarditis in humans and mice are reportedly milder and are more localized than those seen in coxsackievirus myocarditis [8, 9, 14]. Kotaka et al. reported that murine influenza myocarditis was histologically mild and brief in duration compared to coxsackievirus B3 myocarditis [64]. Electron microscopic findings of the heart from a murine influenza myocarditis model showed many infiltrating lymphocytes directly attached to the cardiac myocytes. Nonetheless, the affinity of the influenza virus for cardiac myocytes appears to be low.

Pan et al. investigated the molecular mechanism of myocarditis associated with the influenza virus and revealed the importance of trypsin induction and increased production of matrix metalloproteinase (MMP) and proinflammatory cytokines in the pathogenesis of acute myocarditis [65–68]. Pan et al. also showed that inhibition of trypsin suppressed viral replication, upregulated of MMPs and cytokines, and significantly improved the cardiac function of mice infected with influenza A virus [65–68]. Teijaro et al. revealed immune cell infiltration and cytokine production as distinct events, both of which are orchestrated by endothelial cells [68]. Beside the direct effect of influenza virus infection, proinflammatory cytokines and endothelial cell dysfunction are thought to contribute to the pathogenesis of severe clinical features, including severe cardiac dysfunction and encephalopathy in patients infected with influenza virus [65–68].

Calore et al. observed perivascular hemorrhage of the brain in five of six autopsies of H1N1pdm2009 cases; focal myocarditis was also observed in one case [45]. They suggested that hemorrhagic lesions in the brain might be due to vascular lesions or to an increase in endothelial permeability. Edler et al. demonstrated that an autopsy of a fulminant myocarditis case showed small patch-shaped hemorrhages on the top of the heart and a florid myocarditis with marked mixed-cell infiltrates; H1N1pdm2009 virus was detected in the brain and heart by RT-PCR [53]. RT-PCR from the myocardium showed positive results in four of the patients surveyed in the present paper. Thus, although the pathogenesis of influenza-associated myocarditis remains unclear, the literature suggests that endothelial dysfunction may be important in the pathogenesis of myocarditis and encephalopathy associated with influenza virus.

6. Diagnosis of Myocarditis Associated with Influenza A Virus

In the present paper, chest pain or worsening dyspnea was a common symptom in patients with myocarditis associated with H1N1pdm2009. Cardiac symptoms (e.g., dyspnea, cough, palpitation, and impaired consciousness) developed from the first sick day to the third sick day in 51% of patients. On the other hand, cardiac dysfunction reportedly developed after recovery from flu-like symptoms in two patients. Since cardiac dysfunction progressed rapidly in H1N1pdm2009 myocarditis, early diagnosis and prompt treatment of acute myocarditis with heart failure are required in patients with influenza infection during the pandemic season.

The ECG is a sensitive and convenient tool for diagnosis of myocarditis. ST elevation, T inversion, and conduction block are frequently observed. The ECG and echocardiogram must be repeated for the diagnosis of myocarditis in patients with suspected myocarditis; ECG monitoring is also useful to detect fatal arrhythmias [7, 8]. Myocarditis can be confirmed by observation of transient wall thickening, reduced wall motion, and reduced cardiac chamber size in addition to pericardial effusion on echocardiography [8, 76]. Erden et al. reported that tissue Doppler echocardiography is useful to detect subclinical cardiac dysfunction [77]. It is also important to perform echocardiography to distinguish fulminant myocarditis, which is a lethal disease, from acute myocarditis. Felker et al. reported that patients with fulminant myocarditis had near normal diastolic dimensions with increased septal thickness, while those with acute myocarditis had increased diastolic dimensions with normal septal thickness [11].

Myocarditis is confirmed by the findings of transient elevation of creatinine kinase (CPK), the MB form of creatinine kinase (CPK-MB), and cardiac troponin. Brown et al. reported the usefulness of troponin as a diagnostic test in patients in the pediatric emergency department who report chest pain, although troponin is not useful for excluding cardiac ischemic disease in adults [78]. Erden et al. and Sahin et al. reported acute myocarditis mimicking acute myocardial infarction associated with H1N1pdm2009 infection, with chest pain and ST elevation, suggesting that coronary artery disease should be excluded in cases with severe chest pain [35, 42]. Coronary artery disease was excluded by coronary study in 64% of the adults in this paper. For even more definitive diagnosis, endomyocardial biopsy should be performed after the coronary lesion has been excluded, although it is not essential for the clinical diagnosis of myocarditis. However, even if the results of cardiac biopsy are negative, the presence of myocarditis cannot be excluded due to the possibility of sampling error. Baccouche et al. reported that CMR and endomyocardial biopsy have good diagnostic performance as single techniques in patients with Troponin-I-positive acute chest pain in the absence of coronary artery disease [74]. Liu and Yan observed that CMR, a new technique, is helpful for the detection of myocarditis, because CMR can visualize the entire myocardium [75]. Gutbertet et al. reported that although CMR imaging may be helpful in noninvasively detecting intramyocardial inflammation, it fails to detect viral persistence [73]. Takeuchi et al. reported that MRI might be more useful than invasive cardiac biopsy for diagnosing H1N1pdm2009 myocarditis and for estimating the activity and severity of inflammation [49]. Mavrogeni and Manoussakis recommended CMR for its sensitivity in detecting pericardial/myocardial involvement during H1N1pdm2009 infection, especially if echocardiographic evaluation is negative [55]. Thus, CMR may be useful for the diagnosis of influenza virus myocarditis, because the affinity of influenza virus for cardiac myocytes appears to be low, identifying the intramyocardial inflammation of influenza myocarditis.

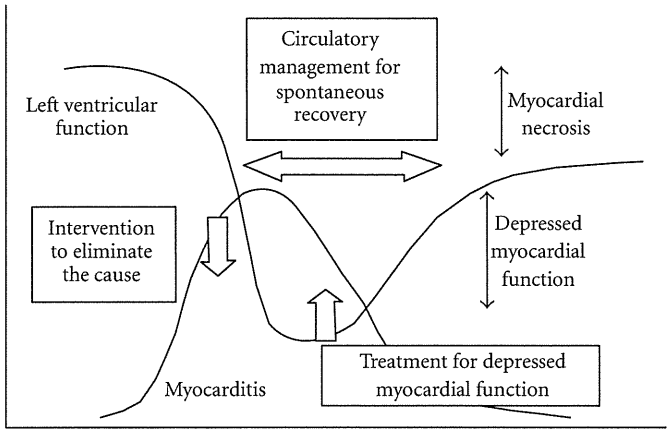

FIGURE 1: Course of cardiac dysfunction and timing of intervention in myocarditis (Guidelines for Diagnosis and Treatment of Myocarditis (JCS2009)).

Usually viral infection is diagnosed if the viral antibody titer is at least four times higher in an acute phase serum sample than that in a sample obtained in the remission phase (with samples collected at least two weeks apart). Although diagnosis of the pathogen in myocarditis is difficult, good diagnostic methods, including a rapid diagnostic test for influenza virus and an RT-PCR assay for influenza H1N1pdm2009 virus from a nasal swab, were already available during the 2009 pandemic. In fact, the prevalence of these new diagnostic methods may be one of the reasons why the number of case reports of myocarditis increased in this pandemic. RT-PCR of the myocardium is more useful for identifying the genomes of viruses causing myocarditis than other methods and showed positive results in 4 patients in our paper [44, 51, 53, 58].

7. Treatment of Myocarditis Associated with Influenza

The course of cardiac dysfunction and timing of intervention are described in the Guidelines for Diagnosis and Treatment of Myocarditis of the Japan Circulation Society (JCS2009) and are shown in Figure 1. Myocarditis is treated in three ways: (1) intervention to eliminate the cause, (2) intervention to improve hemodynamic compromise, and (3) intervention for cardiac dysfunction [8]. To eliminate the cause, forty-one (85%) of the myocarditis patients in our survey were treated with neuraminidase inhibitors. Treatment with neuraminidase inhibitors is also recommended by the Japanese Association of Infection for all patients infected with influenza [29]. The low-case fatality rate in Japan could be a result of aggressive early intervention with antiviral drugs, such as oseltamivir and zanamivir [28, 29]. Morioka et al. reported no cases of influenza-associated encephalopathy or myocarditis in 44 infants aged <3 months treated with oseltamivir for H1N1pdm2009 infection in Japan [30] and therefore recommended oseltamivir as safe and efficacious for use in infants <3 months of age. Use of immunosuppressive therapy is controversial for both acute myocarditis and influenza infection [8, 24, 27–30, 79, 80]. High-dose steroids are not recommended, because of unproven benefit and potentially harmful effect on influenza infection.

The first therapy for myocarditis patients with heart failure is supportive intervention in potentially fatal cases. The recent application of PCPS and/or IABP in serious cases of viral myocarditis has yielded good outcomes. LVAD or PCPS was used in 10 cases, and IABP was used in 11 cases in this paper. Extracorporeal lung assist (ECMO) was used in 12 cases. These mechanical circulatory devices can be used to decrease mortality and as a bridge to transplantation. Seventeen H1N1pdm2009 myocarditis patients were treated with mechanical circulatory support, thirteen (76%) of whom survived. On the other hand, in the national survey of fulminant myocarditis in Japan, 30 of 52 patients (57.7%) survived without antiviral treatment [10]. Because of the nature of the study design, it was difficult to show that the neuraminidase inhibitors significantly improved the survival rate of patients with fulminant myocarditis associated with influenza. Based on our survey, we recommend that patients with fulminant myocarditis be treated with a combination of neuraminidase inhibitors (to eliminate the causative virus) and mechanical circulatory support (to treat depressed myocardial function).

8. Conclusion

We reviewed the details of 58 cases of myocarditis associated with H1N1pdm2009 and found a high prevalence of fulminant myocarditis (36/58, 62%) among them; 14 of these 58 patients died. Diagnosis and treatment during this pandemic were facilitated by improved diagnostic methods (e.g., rapid diagnostic tests, RT-PCR for influenza virus, echocardiogram, and CMR) and by the ready availability of treatment with neuraminidase inhibitors and mechanical circulatory support. We stress the need for increased awareness of influenza-associated myocarditis. Such knowledge will facilitate earlier diagnosis and treatment of this potentially fatal complication during future influenza pandemics.

Acknowledgment

This paper was supported in part by a research grant for intractable diseases from the Ministry of Health, Labour and Welfare, Tokyo, Japan.

References

[1] S. H. Rezkalla and R. A. Kloner, "Influenza-related viral myocarditis," *Wisconsin Medical Journal*, vol. 109, no. 4, pp. 209–213, 2010.

[2] T. Kuiken and J. K. Taubenberger, "Pathology of human influenza revisited," *Vaccine*, vol. 26, supplement 4, pp. D59–D66, 2008.

[3] K. Kumar, M. Guirgis, S. Zieroth et al., "Influenza myocarditis and myositis: case presentation and review of the literature," *Canadian Journal of Cardiology*, vol. 27, no. 4, pp. 514–522, 2011.

[4] M. A. Mamas, D. Fraser, and L. Neyses, "Cardiovascular manifestations associated with influenza virus infection," *International Journal of Cardiology*, vol. 130, no. 3, pp. 304–309, 2008.

[5] R. Oseasohn, L. Adelson, and M. Kaji, "Clinicopathologic study of thirty-three fatal cases of Asian influenza," *The New England journal of medicine*, vol. 260, no. 11, pp. 509–518, 1959.

[6] Y. C. Hsieh, T. Z. Wu, D. P. Liu, P. L. Shao, L. Y. Chang, C. Y. Liu et al., "Influenza pandemics: past, present and future," *Journal of the Formosan Medical Association*, vol. 105, pp. 1–6, 2006.

[7] T. Vikerfors, A. Stjerna, P. Olcen, R. Malmcrona, and L. Magnius, "Acute myocarditis. Serologic diagnosis, clinical findings and follow-up," *Acta Medica Scandinavica*, vol. 223, no. 1, pp. 45–52, 1988.

[8] JCS Joint Working Group, "Guidelines for diagnosis and treatment of myocarditis (JCS, 2009): digest version," *Circulation Journal*, vol. 75, pp. 734–743, 2011.

[9] M. Sekiguchi, M. Hiroe, and N. Hiramitsu, "Natural history of acute viral or idiopathic myocarditis: a clinical and endomyocardial biopsy follow-up," in *New Concepts of Viral Heart Disease*, H. P. Schultheiss, Ed., pp. 33–50, Springer, Berlin, Germany, 1988.

[10] N. Aoyama, T. Izumi, K. Hiramori et al., "National survey of fulminant myocarditis in Japan: therapeutic guidelines and long-term prognosis of using percutaneous cardiopulmonary support for fulminant myocarditis (special report from a Scientific Committee)," *Circulation Journal*, vol. 66, no. 2, pp. 133–144, 2002.

[11] G. M. Felker, J. P. Boehmer, R. H. Hruban et al., "Echocardiographic findings in fulminant and acute myocarditis," *Journal of the American College of Cardiology*, vol. 36, no. 1, pp. 227–232, 2000.

[12] N. E. Bowles, J. Ni, D. L. Kearney et al., "Detection of viruses in myocardial tissues by polymerase chain reaction: evidence of adenovirus as a common cause of myocarditis in children and adults," *Journal of the American College of Cardiology*, vol. 42, no. 3, pp. 466–472, 2003.

[13] A. L. P. Caforio, F. Calabrese, A. Angelini et al., "A prospective study of biopsy-proven myocarditis: prognostic relevance of clinical and aetiopathogenetic features at diagnosis," *European Heart Journal*, vol. 28, no. 11, pp. 1326–1333, 2007.

[14] H. Koide, Y. Kitaura, H. Deguchi, A. Ukimura, K. Kawamura, and K. Hirai, "Genomic detection of enteroviruses in the myocardium. Studies on animal hearts with coxsackievirus B3 myocarditis and endomyocardial biopsies from patients with myocarditis and dilated cardiomyopathy," *Japanese Circulation Journal*, vol. 56, no. 10, pp. 1081–1093, 1992.

[15] A. Montcriol, S. Wiramus, A. Ribeiri et al., "Successful management of Influenza A associated fulminant myocarditis: mobile circulatory support in intensive care unit: a case report," *Cases Journal*, vol. 1, no. 1, article 46, 2008.

[16] D. L. Moore, W. Vaudry, D. W. Scheifele et al., "Surveillance for influenza admissions among children hospitalized in Canadian immunization monitoring program active centers, 2003-2004," *Pediatrics*, vol. 118, no. 3, pp. e610–e619, 2006.

[17] P. C. McGovern, S. Chambers, E. A. Blumberg et al., "Successful explantation of a ventricular assist device following fulminant influenza type A-associated myocarditis," *Journal of Heart and Lung Transplantation*, vol. 21, no. 2, pp. 290–293, 2002.

[18] M. Miura, Y. Asaumi, Y. Wada et al., "A case of influenza subtype A virus-induced fulminant myocarditis: an experience of percutaneous cardio-pulmonary support (PCPS) treatment and immunohistochemical analysis," *Tohoku Journal of Experimental Medicine*, vol. 195, no. 1, pp. 11–19, 2001.

[19] H. Onitsuka, T. Imamura, N. Miyamoto et al., "Clinical manifestations of influenza A myocarditis during the influenza epidemic of winter 1998-1999," *Journal of Cardiology*, vol. 37, no. 6, pp. 315–323, 2001.

[20] K. B. Nolte, P. Alakija, G. Oty et al., "Influenza A virus infection complicated by fatal myocarditis," *American Journal of Forensic Medicine and Pathology*, vol. 21, no. 4, pp. 375–379, 2000.

[21] C. M. Proby, D. Hackett, S. Gupta, and T. M. Cox, "Acute myopericarditis in influenza A infection," *Quarterly Journal of Medicine*, vol. 60, no. 233, pp. 887–892, 1986.

[22] E. Engblom, T. O. Ekfors, and O. H. Meurman, "Fatal influenza A myocarditis with isolation of virus from the myocardium," *Acta Medica Scandinavica*, vol. 213, no. 1, pp. 75–78, 1983.

[23] J. Karjalainen, M. S. Nieminen, and J. Heikkila, "Influenza A1 myocarditis in conscripts," *Acta Medica Scandinavica*, vol. 207, no. 1-2, pp. 27–30, 1980.

[24] Writing Committee of the WHO Consultation on Clinical Aspects Of Pandemic (H1N1) 2009 Influenza, "Clinical aspects of pandemic 2009 influenza A, (H1N1) virus infection," *The New England Journal of Medicine*, vol. 362, pp. 1708–1719, 2010.

[25] Centers for Disease Control and Prevention (CDC), "Patients hospitalized with 2009 pandemic influenza A, (H1N1)—New York City, May 2009," *Morbidity and Mortality Weekly Report*, vol. 58, pp. 1436–1440, 2010.

[26] Y. Itoh, K. Shinya, M. Kiso et al., "In vitro and in vivo characterization of new swine-origin H1N1 influenza viruses," *Nature*, vol. 460, no. 7258, pp. 1021–1025, 2009.

[27] M. D. V. Kerkhove, K. A. Vandemaele, V. Shinde et al., "Risk factors for severe outcomes following 2009 influenza A, (H1N1) infection: a global pooled analysis," *PLoS Medicine*, vol. 7, pp. 1–12, 2011.

[28] The Ministry of Health, Labor, Tokyo, Japan, "A National survey of influenza patients in the last winter in Japan (in Japanese)," http://www.mhlw.go.jp/bunya/kenkou/kekkaku-kansenshou04/pdf/110210-03.pdf, 2011.

[29] N. Sugaya, "Widespread use of neuraminidase inhibitors in Japan," *Journal of Infection and Chemotherapy*, vol. 17, pp. 595–601, 2011.

[30] I. Morioka, S. Nonoyama, K. Tanaka-Taya et al., "Survey of Japanese infants younger than 3 months who were treated with oseltamivir for influenza: safety of oseltamivir treatment,"

Scandinavian Journal of Infectious Diseases, vol. 44, no. 8, pp. 605–609, 2012.

[31] A. Ukimura, T. Izumi, and A. Matsumori, "A national survey on myocarditis associated with the 2009 influenza a (H1N1) pandemic in Japan," *Circulation Journal*, vol. 74, no. 10, pp. 2193–2199, 2010.

[32] E. G. Brooks, C. H. Bryce, C. Avery, C. Smelser, D. Thompson, and K. B. Nolte, "2009 H1N1 fatalities: the new Mexico experience," *Journal of Forensic Sciences*, vol. 57, no. 6, pp. 1512–1518, 2012.

[33] P. N. Mohite, A. F. Popov, A. Bartsch et al., "Successful treatment of novel H1N1 influenza related fulminant myocarditis with extracorporeal life support," *Journal of Cardiothoracic Surgery*, vol. 6, article 164, 2011.

[34] J. U. Rehman, G. Wali, N. M. Sayes, A. Maulawi, M. Aslam, and I. Khalid, "Novel influenza A, (H1N1) virus-induced hemophagocytosis: first case reported in Saudi Arabia," *Annals of Saudi Medicine*, vol. 32, no. 1, pp. 86–89, 2012.

[35] I. Erden, E. C. Erden, H. Ozhan, and C. Basar, "Acute myocarditis mimicking acute myocardial infarction associated with pandemic 2009 (H1N1) influenza A virus," *Cardiology Journal*, vol. 18, no. 5, pp. 552–555, 2011.

[36] O. Adedayo, N. Iheonunekwu, and D. Jefferson, "Acute fulminant myocarditis and the 2009 pandemic influenza A virus (H1N1)," *West Indian Medical Journal*, vol. 60, no. 2, pp. 217–219, 2011.

[37] A. F. Lee, D. A. Chiasson, J. F. Smythe, and S. Sanatani, "Recurrent congestive heart failure in a child due to probable myocarditis," *Pediatric Cardiology*, vol. 33, no. 1, pp. 176–181, 2012.

[38] K. Chan, D. Meek, and I. Chakravorty, "Unusual association of ST-T abnormalities, myocarditis and cardiomyopathy with H1N1 influenza in pregnancy: two case reports and review of the literature," *Journal of Medical Case Reports*, vol. 5, volume 314, 2011.

[39] S. K. Khambekar, S. Harden, and S. Corbett, "Influenza A (H1N1) and myocarditis," *Heart*, vol. 97, no. 19, article 1630, 2011.

[40] Y. C. Liao, Y. C. Hsieh, W. C. Chang, J. L. Huang, C. T. Ting, and T. J. Wu, "Fulminant myocarditis in an adult with 2009 pandemic influenza A (H1N1 influenza) infection," *Journal of the Chinese Medical Association*, vol. 74, no. 3, pp. 130–133, 2011.

[41] R. N. Khouzam, C. Parizianu, A. M. Hafiz, S. Chawla, and R. Schwartz, "Fulminant myocarditis associated with novel H1N1 influenza A," *Heart and Lung*, vol. 40, no. 6, pp. 566–568, 2011.

[42] D. Y. Şahin, M. Demir, B. Kurtaran, and A. Usal, "A case of myocarditis mimicking acute coronary syndrome associated with H1N1 influenza A virus infection," *Turk Kardiyoloji Dernegi Arsivi*, vol. 38, no. 8, pp. 572–575, 2010.

[43] J. A. Wiegand, C. Torgersen, S. Bloechlinger, J. Takala, and M. W. Dünser, "Influenza A(H1N1) infection and severe cardiac dysfunction in adults: a case series," *Wiener Klinische Wochenschrift*, vol. 123, no. 3-4, pp. 120–123, 2011.

[44] G. Gdynia, P. Schnitzler, E. Brunner et al., "Sudden death of an immunocompetent young adult caused by novel (swine origin) influenza A/H1N1-associated myocarditis," *Virchows Archiv*, vol. 458, no. 3, pp. 371–376, 2011.

[45] E. E. Calore, D. E. Uip, and N. M. Perez, "Pathology of the swine-origin influenza A (H1N1) flu," *Pathology Research and Practice*, vol. 207, no. 2, pp. 86–90, 2011.

[46] A. N. Pavlidis, A. K. Giannakopoulos, P. G. Danias, and A. J. Manolis, "A case of acute myocarditis caused by pandemic (H1N1) 2009 influenza virus," *Journal of the Formosan Medical Association*, vol. 109, no. 11, pp. 857–858, 2010.

[47] T. Oda, H. Yasunaga, Y. Tsutsumi et al., "A child with influenza A (H1N1)-associated myocarditis rescued by extracorporeal membrane oxygenation," *Journal of Artificial Organs*, vol. 13, no. 4, pp. 232–234, 2010.

[48] D. Penela, A. Regueiro-Cueva, J. T. Ortiz-Pérez, and M. Sitges, "Myocarditis associated with pandemic influenza A(H1N1)," *Revista Espanola de Cardiologia*, vol. 63, no. 11, pp. 1386–1387, 2010.

[49] I. Takeuchi, R. Imaki, T. Inomata, K. Soma, and T. Izumi, "MRI is useful for diagnosis of H1N1 fulminant myocarditis," *Circulation Journal*, vol. 74, no. 12, pp. 2758–2759, 2010.

[50] N. Asai, Y. Ohkuni, A. Komatsu et al., "A case of asthma-complicated influenza myocarditis," *Journal of Infection and Chemotherapy*, vol. 17, no. 3, pp. 429–432, 2011.

[51] T. Komai, G. Nakazawa, S. Asai, and Y. Ikari, "Fatal fulminant myocarditis associated with novel influenza A (H1N1) infection," *European Heart Journal*, vol. 32, no. 3, article 283, 2011.

[52] D. Calderaro, S. de Souza Dos Santos, A. C. Tonacio et al., "Acute myocarditits in H1N1 influenza a virus infection," *Revista da Associacao Medica Brasileira*, vol. 56, no. 4, p. 394, 2010.

[53] C. Edler, A. Klein, A. Gehl, C. Ilchmann, S. Scherpe, and M. Schrot, "The new influenza A (H1N1/09): symptoms, diagnostics, and autopsy results," *International Journal of Legal Medicine*, vol. 125, no. 2, pp. 157–161, 2011.

[54] R. M. Bramante, A. Cirilli, and C. C. Raio, "Point-of-care sonography in the emergency department diagnosis of acute H1N1 influenza myocarditis," *Journal of Ultrasound in Medicine*, vol. 29, no. 9, pp. 1361–1364, 2010.

[55] S. Mavrogeni and M. N. Manoussakis, "Myocarditis as a complication of influenza A (H1N1): evaluation using cardiovascular magnetic resonance imaging," *Hellenic Journal of Cardiology*, vol. 51, no. 4, pp. 379–380, 2010.

[56] S. Haessler, A. Paez, M. Rothberg, and T. Higgins, "2009 pandemic H1N1-associated myocarditis in a previously healthy adult," *Clinical Microbiology and Infection*, vol. 17, no. 4, pp. 572–574, 2011.

[57] E. R. Gross, J. W. Gander, A. Reichstein, R. A. Cowles, C. J. H. Stolar, and W. Middlesworth, "Fulminant pH1N1-09 influenza-associated myocarditis in pediatric patients," *Pediatric Critical Care Medicine*, vol. 12, no. 2, pp. e99–e101, 2011.

[58] A. Bratincsák, H. G. El-Said, J. S. Bradley, K. Shayan, P. D. Grossfeld, and C. R. Cannavino, "Fulminant myocarditis associated with pandemic H1N1 influenza A virus in children," *Journal of the American College of Cardiology*, vol. 55, no. 9, pp. 928–929, 2010.

[59] T. W. Weiss, K. H. Stensaeth, and J. Eritsland, "Myocarditis in a juvenile patient with influenza A virus infection," *European Heart Journal*, vol. 31, no. 3, p. 277, 2010.

[60] S. S. Martin, C. L. Hollingsworth, S. G. Norfolk, C. R. Wolfe, and J. W. Hollingsworth, "Reversible cardiac dysfunction associated with pandemic 2009 influenza A(H1N1)," *Chest*, vol. 137, no. 5, pp. 1195–1197, 2010.

[61] A. G. Randolph, F. Vaughn, R. Sullivan et al., "Pediatric acute lung injury and sepsis investigator's network and the national heart, lung, and blood institute ARDS Clinical Trials Network. Critically ill children during the 2009-2010 influenza pandemic in the United States," *Pediatrics*, vol. 128, pp. e1450–e1458, 2011.

[62] Y. Zheng, Y. He, J. Deng et al., "Hospitalized children with 2009 influenza A (H1N1) infection in Shenzhen, China,

November-December 2009," *Pediatric Pulmonology*, vol. 46, no. 3, pp. 246–252, 2011.

[63] S. Y. Shin, J. H. Kim, H. S. Kim et al., "Clinical characteristics of Korean pediatric patients critically ill with influenza A (H1N1) virus," *Pediatric pulmonology*, vol. 45, no. 10, pp. 1014–1020, 2010.

[64] M. Kotaka, Y. Kitaura, H. Deguchi, and K. Kawamura, "Experimental influenza A virus myocarditis in mice: light and electron microscopic, virologic, and hemodynamic study," *American Journal of Pathology*, vol. 136, no. 2, pp. 409–419, 1990.

[65] H. Y. Pan, H. Yamada, J. Chida et al., "Up-regulation of ectopic trypsins in the myocardium by influenza A virus infection triggers acute myocarditis," *Cardiovascular Research*, vol. 89, no. 3, pp. 595–603, 2011.

[66] S. Wang, T. Q. Le, N. Kurihara et al., "Influenza virus-cytokine-protease cycle in the pathogenesis of vascular hyperpermeability in severe influenza," *Journal of Infectious Diseases*, vol. 202, no. 7, pp. 991–1001, 2010.

[67] S. Wang, T. Q. Le, J. Chida, Y. Cisse, M. Yano, and H. Kido, "Mechanisms of matrix metalloproteinase-9 upregulation and tissue destruction in various organs in influenza A virus infection," *Journal of Medical Investigation*, vol. 57, no. 1-2, pp. 26–34, 2010.

[68] J. R. Teijaro, K. B. Walsh, S. Cahalan et al., "Endothelial cells are central orchestrators of cytokine amplification during influenza virus infection," *Cell*, vol. 146, pp. 980–991, 2011.

[69] S. A. Huber, J. Kupperman, and M. K. Newell, "Hormonal regulation of CD4+ T-cell responses in coxsackievirus B3-induced myocarditis in mice," *Journal of Virology*, vol. 73, no. 6, pp. 4689–4695, 1999.

[70] A. Angelini, V. Calzolari, F. Calabrese et al., "Myocarditis mimicking acute myocardial infarction: role of endomyocardial biopsy in the differential diagnosis," *Heart*, vol. 84, no. 3, pp. 245–250, 2000.

[71] M. Noutsias, B. Seeberg, H. P. Schultheiss, and U. Kühl, "Expression of cell adhesion molecules in dilated cardiomyopathy: evidence for endothelial activation in inflammatory cardiomyopathy," *Circulation*, vol. 99, no. 16, pp. 2124–2131, 1999.

[72] I. Kindermann, M. Kindermann, R. Kandolf et al., "Predictors of outcome in patients with suspected myocarditis," *Circulation*, vol. 118, no. 6, pp. 639–648, 2008.

[73] M. Gutberlet, B. Spors, T. Thoma et al., "Suspected chronic myocarditis at cardiac MR: diagnostic accuracy and association with immunohistologically detected inflammation and viral persistence," *Radiology*, vol. 246, no. 2, pp. 401–409, 2008.

[74] H. Baccouche, H. Mahrholdt, G. Meinhardt et al., "Diagnostic synergy of non-invasive cardiovascular magnetic resonance and invasive endomyocardial biopsy in troponin-positive patients without coronary artery disease," *European Heart Journal*, vol. 30, no. 23, pp. 2869–2879, 2009.

[75] P. P. Liu and A. T. Yan, "Cardiovascular magnetic resonance for the diagnosis of acute myocarditis: prospects for detecting myocardial inflammation," *Journal of the American College of Cardiology*, vol. 45, no. 11, pp. 1823–1825, 2005.

[76] S. Hiramitsu, S. I. Morimoto, S. Kato et al., "Transient ventricular wall thickening in acute myocarditis: a serial echocardiographic and histopathologic study," *Japanese Circulation Journal*, vol. 65, no. 10, pp. 863–866, 2001.

[77] S. Erden, E. C. Erden, H. Özhan et al., "Echocardiographic manifestations of pandemic 2009 (H1N1) influenza a virus infection," *Journal of Infection*, vol. 61, no. 1, pp. 60–65, 2010.

[78] J. L. Brown, D. A. Hirsh, and W. T. Mahle, "Use of troponin as a screen for chest pain in the pediatric emergency department," *Pediatric Cardiology*, vol. 33, no. 2, pp. 337–342, 2012.

[79] A. Frustaci, M. A. Russo, and C. Chimenti, "Randomized study on the efficacy of immunosuppressive therapy in patients with virus-negative inflammatory cardiomyopathy: the TIMIC study," *European Heart Journal*, vol. 30, no. 16, pp. 1995–2002, 2009.

[80] R. Wojnicz, E. Nowalany-Kozielska, C. Wojciechowska et al., "Randomized, placebo- controlled study for immunosuppressive treatment of inflammatory dilated cardiomyopathy: two-year follow-up results," *Circulation*, vol. 104, no. 1, pp. 39–45, 2001.

DOI:10.1111/j.1750-2659.2012.00421.x
www.influenzajournal.com

Original Article

Increased symptom severity but unchanged neuraminidase inhibitor effectiveness for A(H1N1)pdm09 in the 2010–2011 season: comparison with the previous season and with seasonal A(H3N2) and B

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Accepted 1 July 2012. Published Online 16 August 2012.

Background No studies of the clinical symptoms before starting therapy or of the effectiveness of neuraminidase inhibitors (NAIs) have been carried out of the 2009–2010 and 2010–2011 seasons that compare A(H1N1)pdm09 or the three circulating types of influenza virus.

Methods The clinical symptoms and duration of fever (body temperature ≥37·5°C) after the first dose of an NAI (oseltamivir, zanamivir, laninamivir) were analyzed. PCR was carried out for 365 patients with A(H1N1)pdm09 in the 2009–2010 season and for 388 patients with one of the three types of influenza circulating in the 2010–2011 season. IC_{50} for the three NAIs was also analyzed in 51 patients in the 2010–2011 season.

Results The peak body temperature was significantly higher in 2010–2011 than in 2009–2010 for patients under 20 years with A(H1N1)pdm09, and in the 2010–2011 season for children 15 years or younger with A(H1N1)pdm09 than for those with other virus types. The percentage of A(H1N1)pdm09 patients with loss of appetite or fatigue was significantly higher in 2010–2011 than in the previous season. The duration of fever was not affected by the kind of NAI or by age in multiple regression analysis. The percentage of patients afebrile at 48 hours after the first dose of NAI was significantly higher for A(H1N1)pdm09 than for A(H3N2) (laninamivir) or B (oseltamivir and laninamivir).

Conclusion Although the clinical symptoms of A(H1N1)pdm09 were slightly more severe in the 2010–2011 season, the effectiveness of the NAIs remained high in comparison with 2009–2010 and with other types of seasonal influenza.

Keywords A(H1N1)pdm09, clinical symptom, IC_{50}, laninamivir, neuraminidase inhibitor.

Please cite this paper as: Kawai *et al.* (2013) Increased symptom severity but unchanged neuraminidase inhibitor effectiveness for A(H1N1)pdm09 in the 2010–2011 season: comparison with the previous season and with seasonal A(H3N2) and B. Influenza and Other Respiratory Viruses 7(3), 448–455.

Introduction

Influenza A(H1N1)pdm09 was highly prevalent in the 2009–2010 season, with few cases of A(H3N2) or B reported.[1,2] However, all three subtypes (types) spread widely and almost simultaneously in the 2010–2011 winter season.[1,3,4] Little study has been carried out of the differences in the clinical symptoms or the effectiveness of neuraminidase inhibitors (NAIs) between these two seasons for A(H1N1)pdm09 viruses or among the three influenza subtypes. A(H1N1)pdm09 was reported mainly in the autumn (mostly September–December) of the 2009–2010 season, but prevailed in winter (mostly January–March) in the 2010–2011 season, which is similar to the usual influenza season in Japan.[1] Therefore, we thought it would be interesting to determine how the clinical features of A(H1N1)pdm09 might have differed between these two seasons.

We have reported the usefulness of neuraminidase inhibitors (NAIs) almost annually[5–11] and have shown reduced effectiveness of oseltamivir in the 2008–2009 season, when the oseltamivir-resistant (H275Y NA mutation) A(H1N1) viruses were highly prevalent, compared with the previous

season and with zanamivir.[9,10] The new NAI, laninamivir, became available from the 2010–2011 season in Japan.[4,12,13] However, the effectiveness of NAIs, including laninamivir, against various types of influenza viruses, including A(H1N1)pdm09, has not been clinically compared in the same season.

In this report, we compare the clinical symptoms of A(H1N1)pdm09 patients in the 2009–2010 and 2010–2011 seasons and also among the A(H1N1)pdm09, A(H3N2), and B influenzas that were circulating in the 2010–2011 season. We analyzed the duration of fever ≥37·5°C after the first dose of oseltamivir or zanamivir in both seasons and for all three NAIs in the 2010–2011 season.[2,6,8,10] The IC_{50} (50% inhibitory concentration) of the three NAIs was determined for the three types of influenza virus in the 2010–2011 season.[7,9,14]

Methods

Study procedures

Family doctors, pediatricians, and physicians at 13 clinics who belong to the Influenza Study Group of the Japan Physicians Association participated in the study. Patients were enrolled from August 11, 2009 through April 6, 2010 (median: November 11, 2009) in the 2009–2010 season and from November 18, 2010 through May 23, 2011 (median: January 31, 2011) in the 2010–2011 season. Patients who reported to any of our 13 clinics with an influenza-like illness manifesting any two of the following symptoms: body temperature ≥37·5°C, rhinorrhea, sore throat, cough, general fatigue, loss of appetite, or headache were tested by commercial antigen detection kit. From all outpatients with influenza, diagnosed by antigen detection kit and without severe underlying diseases such as chronic obstructive pulmonary disease or chronic heart disease, those who received NAIs within 48 h after the onset of symptoms were registered in this study after providing informed consent.

Oseltamivir has been reported to be related to the neuropsychiatric symptoms of young adults and has been prohibited in Japan, in most cases, for use by patients aged from 10 to 19 years, and zanamivir and laninamivir are not recommended for patients with underlying respiratory disease or children under 5 years. Thus, intravenous peramivir was administered to a few patients. The symptoms of these patients were analyzed, but were excluded from the analysis of the duration of fever. The decision of which NAI to administer, oseltamivir, zanamivir, laninamivir, or peramivir, was left to the discretion of the patient's physician, who followed the above guidelines and patient preference.

Specimens from nasal swabs, throat swabs, nasal aspirates, or blown nasal discharge were subjected to antigen detection and virus isolation. Of the commercially available antigen detection kits based on immunochromatography, Imuno Ace Flu [Touns], QuickNavi-Flu [Denka Seiken], and Capilia FluA + B [Alfresa Pharma] were mainly used.

Oseltamivir (75 mg for adults and for children who weighed ≥37·5 kg and 2 mg/kg for children who weighed <37·5 kg) was taken orally twice per day for five days. Zanamivir (10 mg for adults and for children aged five years or over) was inhaled twice per day for five days. Laninamivir (20 mg for children <10 years old and 40 mg for adults or children 10 years and older) was inhaled at one sitting.[13] No antipyretics were administered, but acetaminophen was used temporarily in the case of emergency.

Age, sex, vaccination status, results of the antigen detection test kit, and body temperature were recorded for all patients. The date and time of the onset of fever, the date and time of administration of the NAI, and the resolution of fever were recorded by the physician, patient, or an attending family member. The first time point at which a patient reported a fever (temperature, 37·5°C) was defined as the time of onset. Patients were asked to measure body temperature at least three times per day (8:00 A.M., 2:00 P.M., and 8:00 P.M.). The time at which a body temperature of <37·5°C was attained and maintained for more than 24 hours was defined as the time the patient became afebrile. The highest body temperature during the course of the disease was also recorded. For clinical symptoms other than fever, the presence or absence of the following symptoms were noted by the doctor when influenza was diagnosed, cough, rhinorrhea, myalgia, loss of appetite, and fatigue.

All data were collected using an Internet-based protocol based on a server located in a secure room at the Gifu City Medical Association.[15] The time from the initial administration of an NAI to the resolution of fever (the duration of fever after the first dose of NAI) was calculated automatically in the SQL database.[6,10] All study-related documents and procedures were approved by the institutional review board at Hara-Doi Hospital.

Influenza virus isolation

Clinical samples for viral isolation were obtained from nasal or pharyngeal swab, nasal aspiration, or self-blown nasal discharge. Samples were suspended in a solution for virus preservation (M4-RT medium) and sent to a central laboratory (Mitsubishi Chemical Medience Corporation) where they were kept at 4°C. The collected samples were cultured with Madin-Darby canine kidney (MDCK) cells at 33°C.

Viral types and subtypes

The type and subtype of A(H3N2) or B were determined by RT-PCR using subtype-specific primers as described.[16] In brief, viral RNA was extracted from the viral culture supernatant, and then cDNA was synthesized using reverse transcriptase. PCR was carried out with cDNA using

primer sets specific for the viral type and subtype. For the A(H1N1)pdm09 virus, the subtype was determined by real-time RT-PCR with a specific primer set and a fluorescent-labeled probe.[17]

Measurement of the IC_{50} of the NA inhibitors

IC_{50} to oseltamivir carboxylate, zanamivir, and laninamivir was determined by a fluorescence-based neuraminidase inhibition assay, as described elsewhere[9,18], with culture supernatants. Laninamivir and zanamivir were provided by Daiichi Sankyo Co., Ltd. Oseltamivir carboxylate was prepared from oseltamivir phosphate extracted from the commercial preparation Tamiflu® (Chugai Pharmaceutical Co., Ltd., Tokyo, Japan).

Statistical analysis

The Student's *t*-test was used for between-group comparisons of the peak body temperature, the duration of fever, age, the time from the onset to the first visit, and IC_{50}. The chi-square test was also performed to compare between-group differences in the percentage of patients. Multiple regression analysis was performed to determine which factors affected the duration of fever, such as age, sex, vaccination status, the peak body temperature, the influenza type or subtype, the drug administered, and the time from the onset to the start of treatment. A P value $<0{\cdot}05$ was considered statistically significant.

Results

Patient characteristics

A total of 442 patients were enrolled in the 2009–2010 season as were 415 in the 2010–2011 season. The complete data of 753 patients with influenza were available for analysis: 365 patients with A(H1N1)pdm09 aged 1 to 78 years old in the 2009–2010 season and 199 patients with A(H1N1)pdm09 aged 1 to 81 years old, 96 patients with A(H3N2) aged 1–74 years old, and 93 patients with B aged 3-66 years old in the 2010–2011 season. The clinical characteristics of the patients are summarized in Table 1.

The mean age was significantly higher for A(H1N1)pdm09 in the 2010–2011 season (25·7 ± 18·4 years) than in the 2009–2010 season (19·0 ± 13·6 years, $P < 0{\cdot}001$) and for A(H3N2) and B in the 2010–2011 season (19·2 ± 19·5 years, $P < 0{\cdot}01$, and 14·9 ± 11·9 years, $P < 0{\cdot}001$, respectively). More female than male patients had influenza B. No significant differences were found in vaccination status or time from the onset to the first visit at a clinic.

Peak body temperature

No significant differences in peak body temperature were found in the age group analysis or for adults over 15 years. However, in children 15 years or younger, the peak body temperature was significantly higher in A(H1N1)pdm09 in the 2010–2011 season (39·3 ± 0·6°C) than in A(H1N1)pdm09 in the 2009–2010 season (39·1 ± 0·7°C, $P < 0{\cdot}05$) and in A(H3N2) and B (39·0 ± 0·7°C, $P < 0{\cdot}01$ and 38·9 ± 0·5°C, $P < 0{\cdot}001$, respectively). (Table 1)

In comparison with the peak body temperature to A(H1N1)pdm09 in both seasons of patient groups 0–9, 10–19, 20–39, and 40 years or over, the temperatures of the 0–9 and 10–19 years' age groups ($P < 0{\cdot}01$ and $P < 0{\cdot}05$, respectively) were significantly higher in the 2010–2011 than in the 2009–2010 season (Figure 1)

Other clinical symptoms

The symptoms at the first visit to the clinic, except for fever, are shown in Table 2. The percentages of patients with cough, rhinorrhea, myalgia, loss of appetite, and fatigue were significantly higher for patients with A(H1N1)pdm09 infection in the 2010–2011 than in the 2009–2010 season. This was also true for A(H3N2), except for loss of appetite. No significant differences in the percentages were found for A(H3N2) and B infection.

Between-season comparison of children (≤15 years) and adults (>15 years) with A(H1N1)pdm09 showed the percentages of all five symptoms to be significantly higher for adults in the 2010–2011 than in the 2009–2010 season (Figure 2). For children, the percentage of patients with loss of appetite or fatigue was significantly higher in the 2010–2011 than in the 2009–2010 season.

Effectiveness of NAIs

The duration of fever after the first dose of oseltamivir, zanamivir, or laninamivir is shown for 365 patients in 2009–2010 and 374 patients in 2010–2011 season. (Table 3) Fourteen patients (5 with A(H1N1)pdm09, 7 with A(H3N2), and 2 with B) to whom peramivir was administered in the 2010–2011 season were excluded from this analysis.

The duration tended to be shorter for A(H1N1)pdm09 in both seasons than for A(H3N2) or B in the 2010–2011 season. No significant differences in the duration were found among oseltamivir, zanamivir, and laninamivir for A(H1N1)pdm09, A(H3N2), and B in the 2010–2011 season. For A(H1N1)pdm09 infection, the duration of fever after starting oseltamivir or zanamivir therapy was slightly, but not significantly, longer in the 2010–2011 season than in the 2009–2010 season.

Multiple regression analysis that included the type of virus and the peak body temperature showed significant relationships with the duration of fever ($P = 0{\cdot}00055$ and 0·00033, respectively). No significance was found for the duration of fever after the first dose of an NAI with the NAI administered, age, sex, vaccination status, or the time from the onset to the start of treatment (Table 4).

Table 1. Baseline clinical characteristics and peak body temperature of patients 15 years or younger and over 15 years

	2009–2010	2010–2011			*P* value between			
	A(H1N1) pdm09 (a)	A(H1N1) pdm09 (b)	A(H3N2) (c)	B (d)	(a) and (b)	(b) and (c)	(c) and (d)	(b) and (d)
Number of patients	365	199	96	93				
Age, mean years ± SD (range)	19·0 ± 13·6 (1–78)	25·7 ± 18·4 (1–81)	19·2 ± 19·5 (1–74)	14·9 ± 11·9 (3–66)	<0·001	<0·01	NS	<0·001
Male/female	188/177	105/94	58/38	39/54	NS	NS	<0·05	NS
Vaccination* Positive/negative/unknown	74/286/5	45/151/3	31/58/7	27/60/6	NS	NS	NS	NS
Time from the onset To the first visit at clinic (hours)	16·3 ± 11·3	15·4 ± 10·8	15·3 ± 10·8	16·5 ± 11·2	NS	NS	NS	NS
Peak body temperature (°C)	39·0 ± 0·7	39·0 ± 0·7	38·9 ± 0·7	38·9 ± 0·5	NS	NS	NS	NS
≤15 years (*n*)	39·1 ± 0·7 (200)	39·3 ± 0·6 (74)	39·0 ± 0·7 (66)	38·9 ± 0·5 (66)	<0·05	<0·01	NS	<0·001
>15 years (*n*)	38·8 ± 0·6 (165)	38·9 ± 0·7 (125)	38·7 ± 0·7 (30)	38·9 ± 0·5 (27)	NS	NS	NS	NS

*Vaccination for seasonal influenza.
() number of patients.

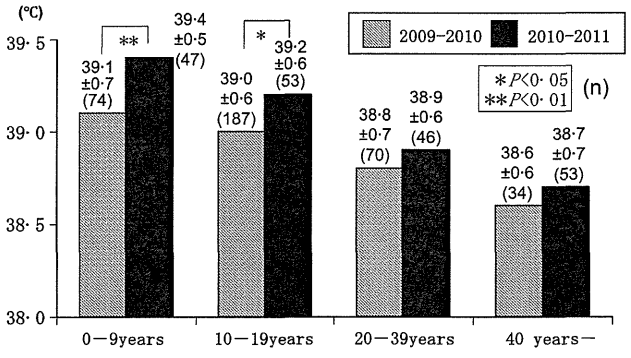

Figure 1. The peak body temperature (°C) of patients with A(H1N1)pdm09 in the 2009–2010 and 2010–2011 seasons, by age. The peak body temperature was significantly higher in the 2010–2011 than the 2009–2010 seasons in the 0–9 and 10–19 years' age groups.

There was no significant difference between the two seasons in the percentage of patients with A(H1N1)pdm09 afebrile at 48 hours after the first dose of oseltamivir or zanamivir (Figure 3).

In the 2010–2011 season, the percentage of patients afebrile at 48 hours after the first dose of laninamivir was significantly higher for A(H1N1)pdm09 (97·1%) than for A(H3N2) and B (81·8%; $P < 0·01$ and 72·2%; $P < 0·001$, respectively) (Figure 3). The percentage after the first dose of oseltamivir was significantly higher for A(H1N1)pdm09 than for B (96·7% and 80·6%, $P < 0·05$). However, no significant difference of duration from the onset to the first dose of an NAI was found between the afebrile and febrile patient groups at 48 hours after the first dose (afebrile and febrile group: 17·1 ± 11·1 and 19·6 ± 14·9 hours in A(H1N1)pdm09, 16·8 ± 11·2 and 18·2 ± 12·1 hours in A(H3N2), and 19·0 ± 10·6 and 16·1 ± 12·5 hours in B, respectively).

In vitro, the IC_{50}s of zanamivir and laninamivir were significantly lower for A(H1N1)pdm09 (0·86 ± 0·32 and 1·77 ± 0·78 nm, respectively) than for A(H3N2) (1·94 ± 0·43 and 3·9 ± 1·6 nm, respectively) or B (12·3 ± 4·0 and 21·3 ± 6·9 nm, respectively). (Table 5) The IC_{50} of oseltamivir was lowest for A(H3N2) (0·74 ± 0·13 nm) and highest for B (44·5 ± 13·6 nm) (Table 5).

Discussion

Cao *et al.* reported that the majority of patients with A(H1N1)pdm09 infection had a mild illness.[19] We also reported that the clinical symptoms of outpatients with A(H1N1)pdm09 infection in the 2009–2010 season tended to be more mild than those of seasonal A(H1N1) in the 2007–2008 and 2008–2009 seasons.[2]

In this study, the peak body temperature was significantly higher in A(H1N1)pdm09 in the 2010–2011 season than in A(H3N2) or B in children 15 years or younger and in A(H1N1)pdm09 in the 2009–2010 season in patients <20 years. The percentage of patients with loss of appetite or fatigue were also higher in the 2010–2011 than in the 2009–2010 season for A(H1N1)pdm09 virus infection in

Table 2. Percentage of patients with each clinical symptoms at first visit to clinics

	2009–2010	2010–2011			*P* value between			
	A(H1N1) pdm09 (a)	A(H1N1) pdm09 (b)	A(H3N2) (c)	B (d)	(a) and (b)	(b) and (c)	(c) and (d)	(b) and (d)
Number of patients	365	199	96	93				
% of patients with each symptom								
Cough	82·7	90·5	82·3	82·8	<0·05	<0·05	NS	NS
Rhinorrhea	49·6	59·8	81·3	71	<0·05	<0·001	NS	NS
Myalgia	27·4	46·7	18·8	25·8	<0·001	<0·001	NS	<0·001
Loss of appetite	23·3	49·2	56·3	44·1	<0·001	NS	NS	NS
Fatigue	44·1	75·4	61·5	62·4	<0·001	<0·05	NS	<0·05

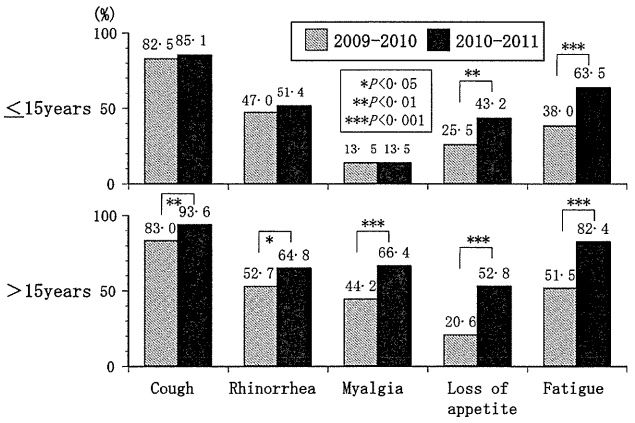

Figure 2. The percentages of the symptoms suffered by patients with A(H1N1) pdm09 infection, by season. The percentage of patients with loss of appetite or fatigue was significantly higher in the 2010–2011 season than in the previous season in children 15 year or younger. The percentage of patients with cough, rhinorrhea, myalgia, loss of appetite, or fatigue was significantly higher in the 2010–2011 season than in the previous season in adults over 15 years.

both the ≤15 years and >15 years' age groups. These results suggest that the severity of symptoms to A(H1N1)pdm09 is increasing as the virus changes from pandemic to seasonal occurrence.

The reason the symptoms to the A(H1N1)pdm09 virus have become slightly more severe is unclear. The percentage of H275Y mutation of A(H1N1)pdm09 in the 2010–2011 season was only 1·1% (2/185) in another of our studies.[4] The virus titer and/or cytokine level may have been increased in this season compared with the previous season. Further study will be necessary. Differences in the season or climate when the A(H1N1)pdm09 was circulating (autumn in the 2009–2010 and winter in the 2010–2011) may also be related to our findings.

We have already reported that oseltamivir was more effective against A(H1N1)pdm09 than against seasonal A(H1N1) in the 2007–2008 and 2008–2009 seasons.[2] We also reported previously that the duration of fever after the first dose of an NAI is significantly correlated, by multiple regression analysis, with the type of virus and peak body

Table 3. The effectiveness of neuraminidaze inhibitors in the 2009–2010 and 2010–2011seasons evaluated by duration of fever

	2009–2010	2010–2011			*P* value between			
Duration of fever after the first dose, hour	A(H1N1) pdm09 (a)	A(H1N1) pdm09 (b)	A(H3N2) (c)	B (d)	(a) and (b)	(b) and (c)	(c) and (d)	(b) and (d)
Oseltamivir	23·1 ± 12·0 (158)	26·5 ± 10·6 (30)	32·0 ± 19·8 (36)	35·7 ± 25·7 (36)	NS	NS	NS	NS
Zanamivir	26·6 ± 15·0 (207)	29·6 ± 18·2 (59)	33·0 ± 22·1 (22)	30·9 ± 16·8 (19)	NS	NS	NS	NS
Laninamivir	n.a	25·0 ± 15·0 (103)	30·9 ± 21·1 (33)	38·5 ± 26·3 (36)		NS	NS	<0·01

() number of patients.
Fourteen patients [5 with A(H1N1)pdm09, 7 with A(H3N2), and 2 with B] to whom peramivir was administered in the 2010–2011 season were excluded from this analysis.

Table 4. Results of multiple regression analysis to determine which factors influenced the duration of fever after the first dose

Factor	*P* value
Age	NS
Sex	NS
Vaccination status	NS
Peak body temperature	0·00033
Influenza type or subtype	0·00055
Drug administered	NS
Time from the onset to the start of treatment	NS

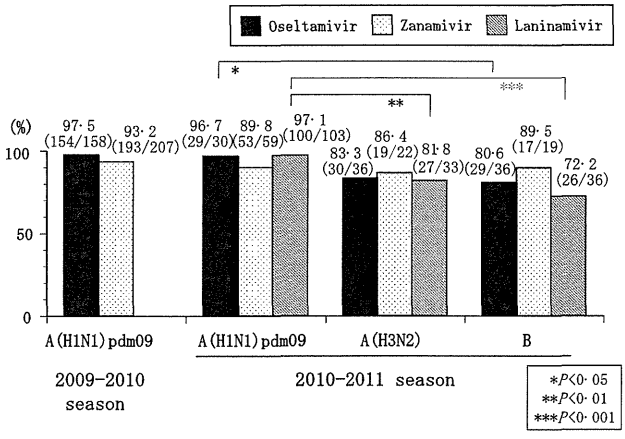

Figure 3. The percentage of patients afebrile at 48 hours after the first dose of each neuraminidase inhibitor. The percentage of patients afebrile at 48 hours after the first dose was significantly higher for A(H1N1)pdm09 than for A(H3N2) (laninamivir) or B (oseltamivir and laninamivir). No significant between-season difference in A(H1N1)pdm09 was found.

temperature, but that there is no correlation with age or the kind of anti-influenza drug.[5] In addition, the effectiveness of vaccination on the duration of fever, as reported in our previous studies, was not confirmed in this study.[5,20]

In this study, the duration of fever and the percentage of patients afebrile at 48 hours after the first dose of oseltamivir or zanamivir did not change significantly from the previous season. However, the duration of fever was significantly shorter for A(H1N1)pdm09 than for B in patients treated with laninamivir, and the percentage of patients afebrile at 48 hours was significantly higher for A(H1N1)pdm09 than for A(H3N2) (laninamivir) or B (oseltamivir and laninamivir).

In our previous study of the 2006–2007 season, the percentages of patients afebrile at 48 hours were 83·1% and 86·7% against influenza A and 55·6% and 80·2% against influenza B for oseltamivir and zanamivir therapy, respectively.[8] In the 2006–2007 season, A(H3N2) was responsible for 90·5% (95/105) of the influenza A cases.[8] The percentage of patients with influenza A(H3N2) afebrile (83·3% and 86·4%, for oseltamivir and zanamivir, respectively) in this study were similar to the data from the 2006–2007 season.

The duration of fever after the first dose of a drug was analyzed to evaluate the clinical effectiveness of these NAIs because it is difficult to evaluate the clinical effectiveness of drugs in outpatient clinics by estimating the mortality rate or incidence of hospitalization. There is a limit to the findings of our study in that it was performed in a general practice setting and not in the context of a rigorous clinical protocol. The body temperature of our outpatients was obtained from reports self-recorded by the patient or a family member. In our previous analysis using this method or virus shedding, oseltamivir was less effective for influenza B than for influenza A and was less effective for A(H1N1) with than without H275Y mutation, especially in children but not so in adults.[9,10] Also, in this study, the duration of fever after oseltamivir therapy tended to be longer in influenza B than in A(H1N1)pdm09 or A(H3N2). However, the difference in the duration between influenza A and B was smaller than in our previous study. The effectiveness of oseltamivir for influenza B compared with A may differ with season. Further study, especially for influenza B, will be necessary.

In this study, we did not compare NAI and non-NAI therapy groups. In Japan, it is unusual to not use an NAI

Table 5. Pre-treatment IC_{50} values for each neuraminidase inhibitor used in the 2010–2011 season

IC_{50} before starting therapy, nm	A(H1N1) pdm09 (a)	A(H3N2) (b)	B (c)	*P* value between (a) and (b)	(b) and (c)	(a) and (c)
Oseltamivir	0·97 ± 0·48 (31)	0·74 ± 0·13 (9)	44·5 ± 13·6 (11)	<0·05	<0·001	<0·001
Zanamivir	0·86 ± 0·32 (31)	1·94 ± 0·43 (9)	12·3 ± 4·0 (11)	<0·001	<0·001	<0·001
Laninamivir	1·77 ± 0·78 (31)	3·9 ± 1·6 (9)	21·3 ± 6·9 (11)	<0·001	<0·001	<0·001

*Duration of fever after the first dose () number of patients.

for patients with influenza diagnosed by commercial antigen detection kit. The usefulness of NAIs is wide, and NAI therapy is supported by the public medical insurance system. We previously reported that the duration of fever was shorter in NAI therapy than in non-NAI therapy in patients with seasonal influenza.[6,12] We have also reported that the usefulness of oseltamivir and zanamivir for A(H1N1)pdm09 is equal to or higher than for seasonal A(H1N1) without H275Y NA mutation.[2]

The severity of the first and second influenza A(H1N1)pdm09 waves was compared in England.[21–23] Keramarou *et al.*[21] reported more hospital admissions (n = 379) and deaths (n = 26) in Wales in the second wave (peaked in late October, 2009) than in the first wave (n = 44 and only one, respectively; peaked in late July, 2009). Higher mortality rates in the second (September–February) than in the first (June–August) wave were also reported by Presanis *et al.*, (0·025% and 0·015% of patients with A(H1N1)pdm09, respectively) and Mytton *et al.* (5·5 and 1·6 deaths per million population, respectively).[22,23] Our results may coincide with these results; however, accurate comparison is difficult because NAIs are more commonly used in Japan than in England. To our knowledge, no comparison of the severity of A(H1N1)pdm09 virus infection in the first or second waves of the 2009–2010 season and the 2010–2011 season has been reported.

Laninamivir octanoate is inhaled, then converted to laninamivir in the lung, and the binding of laninamivir to virus NA is relatively more stable and lasts longer than has been observed for other NAIs.[13,24] In this study, laninamivir was almost equally as effective as oseltamivir or zanamivir, estimated clinically by the duration of fever; nevertheless, the IC_{50} of laninamivir tended to be higher than that of the other NAIs. Kubo, *et al.* recently reported that 6 days after intranasal administration of 236 μg/kg laninamivir octanoate, the concentration of laninamivir in the lungs of mice was maintained about 730-fold the IC_{50} for A(H1N1)pdm09, 77-fold that of A(H3N2), and 70-fold that of B.[22] In another of our studies, the persistence rates of virus culture 4–6 days after the start of laninamivir therapy were 2·3% (2/86) for A(H1N1)pdm09, 10·5% (2/19) for A(H3N2), and 29·4% (5/17) for B in the 2010–2011 season (Unpublished data by Kawai N, Ikematsu H and Kashiwagi S). Thus, laninamivir has been shown to be more effective against A(H1N1)pdm09 than against either A(H3N2) or B in both *in vitro* and *in vivo* studies. In addition, laninamivir is very convenient to use in outpatient clinics because it can be administered in a single sitting.

In conclusion, although the fever of patients with A(H1N1) pdm09 infection improved quickly with NAI therapy in the 2010–2011 season, the clinical symptoms were more severe than in the 2009–2010 season and more severe than for A(H3N2) or B virus infection. It is notable that the effectiveness of oseltamivir and zanamivir for A(H1N1)pdm09 virus infection has not changed since emergence in 2009 and that the effectiveness of laninamivir for A(H1N1)pdm09 was also high. These NAIs should continue to be recommended, especially for A(H1N1)pdm09 virus infection.

Acknowledgements

We thank Drs Osame Tanaka, Shinro Matsuura, Kenichi Kawamura, Satoshi Yamauchi, Ken-ichi Doniwa, and Kunio Kondou for their support in this study.

References

1 Infectious disease Surveillance Center. Weekly reports of influenza virus isolation/detection. Available at https://hasseidoko.mhlw.go.jp/Byogentai/Pdf/data2e.pdf.
2 Kawai N, Ikematsu H, Tanaka O *et al.* Comparison of the clinical symptoms and the effectiveness of neuraminidase inhibitors for patients with pandemic influenza H1N1 2009 or seasonal H1N1 influenza in the 2007–2008 and 2008–2009 seasons. J Infect Chemother 2011; 17:375–381.
3 WHO. Summary review of the 2010–2011 northern hemisphere winter influenza season. Distribution of virus subtypes by influenza transmission zone (October 2010-April 2011). Available at http://www.who.int/influenza/2010_2011_seasonal_review_map_main.jpg.
4 Ikematsu H, Kawai N, Kashiwagi S. In vitro neuraminidase inhibitory activities of four neuraminidase inhibitors against influenza viruses isolated in the 2010-2011 season in Japan. J Infect Chemother. 2012 Feb 28.
5 Kawai N, Ikematsu H, Iwaki N *et al.* Factors influencing the effectiveness of oseltamivir and amantadine for the treatment of influenza: a multicenter study from Japan of the 2002–2003 influenza season. Clin Infect Dis 2005; 40:1309–1316.
6 Kawai N, Ikematsu H, Iwaki N *et al.* A Comparison of the effectiveness of oseltamivir for the treatment of influenza A and influenza B: a Japanese multicenter study of the 2003–2004 and 2004–2005 influenza seasons. Clin Infect Dis 2006; 43:439–444.
7 Kawai N, Ikematsu H, Iwaki N *et al.* Longer virus shedding in influenza B than in influenza A among outpatients treated with oseltamivir. J Infect 2007; 55:267–272.
8 Kawai N, Ikematsu H, Iwaki N *et al.* A comparison of the effectiveness of zanamivir and oseltamivir for the treatment of influenza A and B. J Infect 2008; 56:51–57.
9 Kawai N, Ikematsu H, Iwaki N *et al.* Clinical effectiveness of oseltamivir for influenza A(H1N1) virus with H274Y neuraminidase mutation. J Infect 2009; 59:207–212.
10 Kawai N, Ikematsu H, Hirotsu N *et al.* Clinical effectiveness of oseltamivir and zanamivir for treatment of influenza A virus subtype H1N1 with the H274Y mutation. A Japanese multicenter study of the 2007–2008 and 2008–2009 Influenza Seasons. Clin Infect Dis 2009; 49:1825–1835.
11 Kawai N, Ikematsu H, Iwaki N *et al.* Persistence of pandemic influenza H1N1 virus in young patients after oseltamivir therapy in the 2009-2010 season: a comparison with seasonal H1N1 with or without H275Y mutation. J Infect Chemother 2012; 18:180–186, DOI 10.1007/s10156-011-0314-2.
12 Watanabe A, Chang SC, Kim MJ, Chu DW, Ohashi Y, MARVEL Study Group. Long-acting neuraminidase inhibitor laninamivir

octanoate versus oseltamivir for treatment of influenza: A double-blind, randomized, noninferiority clinical trial. Clin Infect Dis 2010; 51:1167–1175.

13 Ikematsu H, Kawai N. Laninamivir octanoate: a new long-acting neuraminidase inhibitor for the treatment of influenza. Expert Rev Anti Infect Ther 2011; 9:851–857.

14 Boivin G, Goyette N. Susceptibility of recent Canadian influenza A and B virus isolates to different neuraminidase inhibitors. Antiviral Res 2002; 54:143–147.

15 Kawai N, Ikematsu H, Iwaki N *et al.* A prospective, Internet-based study of the effectiveness and safety of influenza vaccination in the 2001–2002 influenza season. Vaccine 2003; 21:4507–4513.

16 Stockton J, Ellis JS, Saville M, Clewley JP, Zambon MC. Multiplex PCR for typing and subtyping influenza and respiratory syncytial viruses. J Clin Microbiol 1998; 36:2990–2995.

17 CDC protocol of realtime RT-PCR for influenza A (H1N1). Available at http://www.who.int/csr/resources/publications/swineflu/realtime-ptpcr/en/index.html.

18 Yamashita M, Tomozawa T, Kakuta M, Tokumitsu A, Nasu H, Kubo S. CS-8958, a prodrug of the new neuraminidase inhibitor R-125489, shows long-acting anti-influenza virus activity. Antimicrob Agents Chemother 2008; 53:186–192.

19 Cao B, Li XW, Mao Y *et al.* Clinical features of the initial cases of 2009 pandemic influenza A (H1N1) virus infection in China. N Engl J Med 2009; 361:2507–2517.

20 Ikematsu H, Takeuchi Y, Rosenlund M *et al.* The post-infection outcomes of influenza and acute respiratory infection in patients above 50 years of age in Japan: an observational study. Influenza Other Respi Viruses 2012; 6:211–217.

21 Keramarou M, Cottrell S, Evans MR *et al.* Two waves of pandemic influenza A(H1N1) 2009 in Wales–the possible impact of media coverage on consultation rates, April-December 2009. Euro Surveill 2011; 16:pii: 19772.

22 Presanis AM, Pebody RG, Paterson BJ *et al.* Changes in severity of 2009 pandemic A/H1N1 influenza in England: a Bayesian evidence synthesis. BMJ 2011; 343:d5408, doi: 10.1136/bmj.d5408.

23 Mytton OT, Rutter PD, Mak M, Stanton EA, Sachedina N, Donaldson LJ. Mortality due to pandemic (H1N1) 2009 influenza in England: a comparison of the first and second waves. Epidemiol Infect 2012; 140:1533–41.

24 Kubo S, Tokumitsu A, Tomozawa T, Kakuta M, Yamashita M. High and continuous exposure of laninamivir, an anti-influenza drug, may work suppressively to generate low-susceptibility mutants in animals. J Infect Chemother 2012; 18:69–74.

J Infect Chemother (2012) 18:529–533
DOI 10.1007/s10156-012-0377-8

ORIGINAL ARTICLE

In vitro neuraminidase inhibitory activities of four neuraminidase inhibitors against influenza viruses isolated in the 2010–2011 season in Japan

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Received: 7 November 2011 / Accepted: 23 January 2012 / Published online: 28 February 2012

Abstract The half maximal inhibitory concentration (IC_{50}) of four neuraminidase inhibitors (NAIs), oseltamivir, zanamivir, laninamivir, and peramivir; was measured using influenza viruses isolated in the 2010–2011 influenza season in Japan. Clinical samples for viral isolation were obtained from nasal aspiration, nasopharyngeal swab, or self-blown nasal discharge and cultured with Madin–Darby canine kidney cells. The type and subtype of H3N2 or B were determined by reverse transcriptase polymerase chain reaction (RT-PCR). For the A(H1N1)pdm09 virus, the subtype was determined by real-time RT-PCR. IC_{50}s to oseltamivir carboxylate, zanamivir, laninamivir, and peramivir were determined by a fluorescence-based neuraminidase inhibition assay. Influenza viruses were isolated from 269 patients. A(H1N1)pdm09, H3N2, and B were isolated from 185, 54, and 30 patients, respectively. The geometric means of IC_{50} for oseltamivir were 0.86 and 0.73 nM to A (H1N1) pdm09, except for the two outlier viruses described below and H3N2, respectively, and 33.12 nM for B. The geometric means of IC_{50} for the other three NAIs were lowest to A(H1N1)pdm09 and highest to B. Two A(H1N1)pdm09 isolates showed very high IC_{50} values for oseltamivir (840 and 600 nM) and peramivir (19 and 24 nM). No isolate showed significantly high IC_{50} values for zanamivir or laninamivir. Continuous surveillance against the emergence or spread of influenza virus with high IC_{50} values for anti-influenza drugs is important.

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Keywords Influenza · Half maximal inhibitory concentration (IC_{50}) · Oseltamivir · Zanamivir · Laninamivir · Peramivir

Introduction

Treating influenza with neuraminidase inhibitors (NAIs) has become the most popular treatment among primary care doctors in Japan. A swine-origin H1N1 strain, A(H1N1) pdm09, was the cause of a pandemic in 2009 [1]. Fortunately, the number of reported influenza-associated deaths was only about 200 in Japan, far fewer than in other countries [1]. The early start of treatment with NAIs, within 48 h of the onset of the influenza symptoms, may have contributed to mitigating symptoms and preventing severe disease.Two NAIs, oseltamivir (Chugai Pharmaceutical Co., Ltd., Tokyo, Japan) and zanamivir (GlaxoSmithKline K.K. Tokyo, Japan), are commonly used in Japanese clinics. The clinical effectiveness of anti-influenza drugs has been confirmed in clinical settings [2–4]. Recently, two new NAIs, laninamivir (Daiichi Sankyo Co., Ltd., Tokyo, Japan) and peramivir (Shionogi & Co., Ltd., Osaka, Japan), were added to the options for influenza treatment in Japan. However, as these various NAIs have been available in the market, drug resistance has become of important clinical concern. An A/H1N1 oseltamivir-resistant strain with a mutation at position 275 of NA was reported in Europe in 2007, and it quickly spread throughout the world [5]. Almost all seasonal A/H1N1 viruses have acquired resistance to oseltamivir worldwide [6]. It has been reported that the H275Y mutant reduces sensitivity to oseltamivir by several hundred-fold in vitro [7]. Reduced clinical effectiveness of oseltamivir to H275Y mutated H1N1 viruses compared to the wild-type H1N1 seasonal influenza virus has been confirmed in the clinical

setting [8, 9]. In addition, the emergence of H275Y mutated A(H1N1)pdm09 with resistance to oseltamivir has been reported [10]. To study the extent of drug resistance, we surveyed the half maximal inhibitory concentration (IC_{50}) of four NAIs, oseltamivir, zanamivir, laninamivir, and peramivir, from influenza viruses isolated in the 2010–2011 influenza season in Japan. The results, including two A(H1N1)pdm09 isolates with significantly high IC_{50} values for oseltamivir and peramivir, but not for zanamivir and laninamivir, are reported.

Materials and methods

Patients

A total of 22 clinics and hospitals from 13 prefectures in Japan participated in this study. Patients were enrolled from 1 November 2010 to 30 April 2011. Samples for viral isolation were collected from patients who showed a positive result by rapid influenza antigen detection kits, based on immunochromatography, with informed consent.

Influenza virus isolation

Clinical samples for viral isolation were obtained from nasal aspiration, nasopharyngeal swab, or self-blown nasal discharge. Samples were suspended with a solution for virus preservation (M4-RT medium, Remel, KS, USA) and sent to a central laboratory (Mitsubishi Chemical Medience Corporation) where they were kept at −80°C. The collected samples were cultured with Madin–Darby canine kidney (MDCK) cells at 33°C.

Viral types and subtypes

The type and subtype of H3N2 or B was determined by amplified DNA size of reverse transcriptase polymerase chain reaction (RT-PCR) using type- and subtype-specific primers as described [11]. In brief, viral RNA was extracted from the clinical sample, then complementary DNA (cDNA) was synthesized using reverse transcriptase. PCR was done with cDNA using primer sets specific for viral type and subtype. For the A(H1N1)pdm09 virus, the subtype was determined by real-time RT-PCR with a specific primer set and a fluorescent-labeled probe (http://www.who.int/csr/resources/publications/swineflu/realtimeptpcr/en/index.html).

Measurement of IC_{50} of NA inhibitors

IC_{50}s to oseltamivir carboxylate, zanamivir, laninamivir, and peramivir were determined by a fluorescence-based neuraminidase inhibition assay with culture supernatants, as described elsewhere [12]. Laninamivir and zanamivir were provided by Daiichi Sankyo Co., Ltd. (Tokyo, Japan). Oseltamivir carboxylate was prepared from oseltamivir phosphate extracted from the commercial preparation Tamiflu® (Chugai Pharmaceutical Co., Ltd., Tokyo, Japan). Peramivir was obtained from the commercially available product (Rapiacta®, Shionogi & Co., Ltd., Osaka, Japan).

Statistical analysis

Difference in age distribution among A(H1N1)pdm09, H3N2, and B patient groups was tested by analysis of variance (ANOVA). Quantitative data were tabulated to provide descriptive summary statistics. Geometric means and 95% confidence intervals (CI) were calculated for IC_{50} values. Box and whisker plots were drawn with log-transformed IC_{50} values by influenza type and subtype. For A(H1N1)pdm09, scatter plots of log-transformed IC_{50} values were made to compare the IC_{50} values of each NAI. P value <0.05 was considered statistically significant. All analyses were performed by SAS® System Release 8.2 (SAS Institute, Cary, NC, USA).

Results

A total of 289 influenza-kit-positive patients were enrolled. Among them, 269 influenza viruses were isolated. Influenza virus A(H1N1)pdm09, H3N2, and B were isolated from 185, 54, and 30 patients, respectively. Age distribution of the patients by virus type and subtype is listed in Table 1. The mean age of the 269 patients who had a virus isolated was 28.1 ± 17.1 years. There was no significant difference in mean ages between males and females. The mean age of A(H1N1)pdm09-positive patients was 30.0 ± 16.2 years, higher than that of H3N2 and B (23.1 ± 18.4 and 21.2 ± 16.5 years, respectively). The difference of age distribution between patients with A(H1N1)pdm09 and H3N2 or B infection was statistically significant ($P = 0.0009$).

The geometric mean of IC_{50} for the four NAIs is listed in Table 2. The geometric mean of IC_{50} for oseltamivir was 0.86 and 0.73 nM to A(H1N1)pdm09, except for the two outlier viruses described below and H3N2, respectively; and 33.12 nM for B. The geometric mean of IC_{50} for the other three NAIs was lowest to A(H1N1)pdm09 and highest to B. The ratio of IC_{50} for B to that of H3N2 for oseltamivir was 45.4 and for zanamivir, laninamivir, and peramivir were 6.8, 6.6, and 6.0, respectively.

The distribution of IC_{50} of the four NAIs is depicted in Fig. 1. The $\log_{10}$ (IC_{50})s of each NAI were distributed in a

Table 1 Distribution of patients by age, sex and virus type

Age group	No. of patients	Males	Females	A(H1N1) pdm09	H3N2	B
0–9	33	14	19	14	13	6
10–19	65	41	24	36	17	12
20–29	54	30	24	43	5	6
30–39	43	24	19	34	6	3
40–49	38	22	16	30	7	1
50–59	25	12	13	22	3	0
60–69	8	2	6	4	3	1
70–79	3	2	1	2	0	1
80+	0	0	0	0	0	0
Total	269	147	122	185	54	30
Mean age ± SD (years)	28.1 ± 17.1	27.5 ± 16.4	28.8 ± 18.0	30.0 ± 16.2	23.1 ± 18.4	21.2 ± 16.5

Data are shown as the number of mean ± standard deviation

Table 2 Half maximal inhibitory concentration (IC_{50}) values of four neuraminidase inhibitors (NAIs) for viral isolates from the 2010–2011 influenza season in Japan

Drug	Geometric mean IC_{50} (nM)		
	A(H1N1)pdm09 (n = 185) Geometric mean (95% CI)	H3N2 (n = 54) Geometric mean (95% CI)	Influenza B (n = 30) Geometric mean (95% CI)
Oseltamivir	0.86 (0.76–0.98)	0.73 (0.65–0.82)	33.12 (28.78–38.09)
Zanamivir	0.73 (0.69–0.78)	1.64 (1.51–1.79)	11.21 (9.98–12.61)
Laninamivir	1.37 (1.27–1.47)	3.22 (2.91–3.56)	21.25 (19.12–23.64)
Peramivir	0.38 (0.34–0.42)	0.66 (0.61–0.71)	3.96 (3.44–4.55)

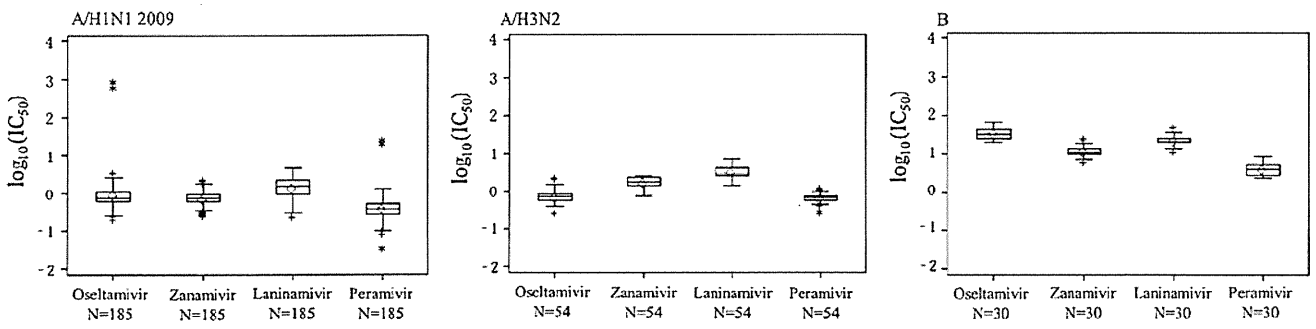

Fig. 1 Half maximal inhibitory concentration (IC_{50}) quartiles of each neuraminidase inhibitor (NAI) for different influenza types. *Diamond* arithmetic mean, *plus symbol* values between 1.5 × IQR and 3 × IQR from UQ/LQ; *asterisk* values above/below 3 × IQR from UQ/LQ, respectively. *IQR* interquartile range, *UQ* 75 percentile, *LQ* 25 percentile

narrow range, except for two viral isolates of A(H1N1)pdm09. The two A(H1N1)pdm09 isolates showed very high IC_{50} values for oseltamivir (840 and 600 nM) and peramivir (19 and 24 nM).

Scatter plots of the log-transformed IC_{50} values of each NAI are shown in Fig. 2. Two isolates showed very high IC_{50} values for oseltamivir but not for zanamivir (Fig. 2a) or laninamivir (Fig. 2b). Two isolates showed high IC_{50} values for both oseltamivir and peramivir (Fig. 2c). No isolate showed a very high IC_{50} value for zanamivir or laninamivir (Fig. 2d). Two isolates showed very high IC_{50} values for peramivir but not for zanamivir (Fig. 2e) or laninamivir (Fig. 2f).

Discussion

In the 2010–2011 season, three influenza strains, A(H1N1) pdm09, H3N2, and B were epidemic in Japan. In this study, A(H1N1)pdm09 was responsible for 68.8% of the isolated viruses. In the 2009–2010 season, almost all clinical isolates were reported to be A(H1N1)pdm09, and patients were mainly 19 years of age and younger. In this study, almost 30% of the patients with A(H1N1)pdm09 were in this age group. The reason for change in the rate of A(H1N1)pdm09 patients in this age group is unknown. For the four NAIs, there was a tendency for the IC_{50} of influenza B virus to be higher than that of A(H1N1)pdm09 and H3N2. The ratio of